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Title: Clinical leishmaniasis in dogs living in the UK

Abstract:

Objective: To investigate the prevalence of leishmaniasis in a canine population in the UK and to describe clinical presentation, clinicopathological abnormalities, therapeutic protocols and outcome in this non-endemic country.

Materials and Methods: Medical records of dogs diagnosed with leishmaniasis at 7 referral centres in the UK were retrospectively reviewed.

Results: The prevalence was between 0.007 and 0.04% with a higher number of cases in southern England. All dogs had a history of travel to or from an endemic country. Lethargy, dermatological signs, decreased appetite and lameness were the most common reasons for presentation. Allopurinol was used alone in the majority of cases.

Clinical significance: Although rare, CanL in the UK should be considered in patients showing compatible clinical signs and with a history of travel to or from endemic areas.

Keywords: Canine, Infectious Disease, Leishmania, UK

29 INTRODUCTION

30 Canine leishmaniasis (CanL) is a systemic zoonotic disease caused by the protozoan *Leishmania infantum*.
31 Infected dogs are the main reservoir of the parasite (Baneth *et al.* 2008) and play an important role in the
32 epidemiology of human visceral (HVL) and cutaneous (HCL) leishmaniasis. Approximately 0.2 to 0.4 and 0.7
33 to 1.2 million HVL and HCL cases, respectively, occur each year. More than 90% of global HVL cases occur
34 in six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil. HCL is more widely distributed
35 with about one-third of cases occurring in each of three epidemiological regions, the Americas, the
36 Mediterranean basin, and western Asia from the Middle East to Central Asia (Alavar *et al.* 2012). CanL is
37 endemic in more than 70 countries worldwide (Solano-Gallego *et al.* 2011) and especially in the
38 Mediterranean areas of Europe (Cyprus, Greece, Albania, Croatia, Italy, Malta, France, Spain and Portugal)
39 (Maia & Cardoso 2015), the Middle East and many tropical and subtropical areas of the world. However, the
40 infection is spreading to non-endemic areas with an increasing number of cases reported in dogs living in
41 North America (Gaskin *et al.* 2002, Duprey *et al.* 2006) and Northern Europe (Shaw *et al.* 2009, Maia &
42 Cardoso 2015). Recent studies have documented the presence of the disease in Germany (Geisweid *et al.*
43 2012), United Kingdom (Shaw *et al.* 2009) and Netherlands (Teske *et al.* 2002). This is probably due to a
44 wider spread of the vector in some of the above areas and especially to a larger numbers of dogs being
45 imported from, or having visited, endemic countries. Since the introduction of the United Kingdom Pet Travel
46 Scheme (PETS) in 2000, the number of dogs travelling into the UK has increased year after year with a total
47 of 411,582 dogs recorded between 2000 and January 2008 (Mencke *et al.* 2011). As a result, the disease has
48 gained importance in the UK, albeit largely limited to the dogs that travel. It is likely that only very little
49 natural transmission occurs in the UK because environmental conditions prevent the viability of the vector
50 (Shaw *et al.* 2009). However, other mechanisms of transmission are possible, including blood transfusion (de
51 Freitas *et al.* 2006), vertical (Rosypal *et al.* 2005, Pangrazio *et al.* 2009, Boggiatto *et al.* 2011, Naucke *et al.*
52 2012, Turchetti *et al.* 2014) and venereal transmission (Diniz *et al.* 2006). Despite the increase in awareness,
53 the prevalence of infected dogs entering the UK is unknown, as no pre-or post-travel testing is required.
54 Furthermore, clinically apparent cases represent the minority of infected dogs in endemic areas (Solano-
55 Gallego *et al.* 2009; Schallig *et al.* 2013), so dogs with subclinical infection that appear healthy may
56 unknowingly be imported.

57 Only one study (Shaw *et al.* 2009) has previously investigated dogs with positive diagnostic tests for CanL in
58 the UK. The objectives of this study were, firstly, to investigate the prevalence of leishmaniasis in a canine
59 population attending referral centres in the UK and, secondly, to describe clinical presentation, most frequent
60 clinicopathological abnormalities, diagnostic investigations, different therapeutic protocols used and outcome
61 of dogs diagnosed with CanL in this non-endemic country.

62

63 **MATERIALS AND METHODS**

64 **Patients and eligibility**

65 Medical records of dogs diagnosed of clinical leishmaniasis in seven different referral centres in the UK
66 (University of Liverpool, University of Bristol, University of Edinburgh, University of Cambridge, Royal
67 Veterinary College of London, Anderson Moores Veterinary Specialists, Animal Health Trust), between
68 January 2005 and January 2014, were retrospectively reviewed. The database of each institution was searched
69 by use of the following terms: leishmaniasis, leishmaniosis, allopurinol, N-methylglucamine antimoniate and
70 miltefosine. Dogs on therapy with allopurinol for other diseases than CanL were excluded. In this way only
71 patients with a final diagnosis of clinical leishmaniasis were selected and then included. The prevalence of
72 the disease was calculated as the percentage of the ratio between the number of cases diagnosed of CanL in
73 the study period at each referral centre and the total canine population that attended the respective centre in
74 the same time period. The study was approved by the Veterinary Research Ethics Committee of the University
75 of Liverpool.

76

77 **Data collection**

78 The diagnosis of CanL was made when there were compatible clinical signs and/or laboratory abnormalities
79 together with detection of the parasites by polymerase chain reaction (PCR) or cytology (from lymph node,
80 bone marrow, spleen or skin), and/or detection of antibodies using an immunofluorescence assay (IFAT) or an
81 enzyme-linked immunosorbent assay (ELISA). Where available, information was reviewed regarding travel
82 history (i.e. country to which the dog had travelled or from where it had been imported), reasons for
83 presentation, physical examination findings, results of diagnostic investigations (e.g. haematology,
84 biochemistry, urinalysis, cytology, serology, and PCR), therapeutic protocol used, and outcome. Dogs were

85 tested for other vector-borne diseases (*Ehrlichia canis*, *Babesia canis*) if a co-infection was suspected.
86 Survival time was defined as time (in days) from first presentation to last re-check or to time of death.

87

88 **Diagnostic investigations**

89 All routine clinicopathological analyses, serology, and real-time quantitative PCR (qPCR) assays were
90 conducted at the respective university or by commercial laboratories. Clinicopathological abnormalities such
91 as anaemia, azotaemia, hypoalbuminaemia were defined when results were outside the reference intervals
92 established by each corresponding laboratory. Proteinuria was diagnosed by an elevated urine protein-to-
93 creatinine ratio (UPCR >0.5) with inactive urinary sediment. Renal azotaemia was defined as increased
94 creatinine with concurrent isosthenuria (1.008-1.012).

95 For serological investigations, the upper reference interval for IFAT was either 1:80 or 1:128, depending on
96 the laboratory, whilst the positive threshold value for ELISA used by all laboratories was 35 ELISA Units
97 (EU). Serological results were classified as low, medium or high positive if IFAT titres were <2-fold, 2- to 4-
98 fold, or >4-fold greater than the threshold positive value indicated by the reference laboratory. ELISA results
99 were classified as mild when <80 EU, moderate when between 80 and 150 EU, and high when >150 EU.
100 Detection and quantification of *Leishmania* kinetoplastic DNA was performed on blood, bone marrow, and/or
101 skin samples by qPCR as previously described (Caldin *et al.* 2004, Solano-Gallego *et al.* 2007, Maia *et al.*
102 2008).

103

104 **Classification of cases**

105 Dogs were classified at the time of diagnosis in different clinical stages according to the Canine Leishmaniasis
106 Working Group (CLWG) guidelines (Paltrinieri *et al.* 2010). Dogs were also classified according to the
107 International Renal Interest Society (IRIS) guidelines, based on measurement of serum creatinine
108 concentration at the first two appointments.

109

110 **Statistical analysis:**

111 Data are reported as median or mean and range (minimum and maximum).

112

113 **RESULTS:**

114 **Patient population**

115 Thirty-eight dogs were included in the study: 14 were diagnosed at the Royal Veterinary College, 7 at the
116 University of Liverpool, 7 at the University of Edinburgh, 3 at the University of Bristol, 3 at the University of
117 Cambridge, 3 at the Animal Health Trust and 1 at Anderson Moores Veterinary Specialists. Median age was
118 4.8 years (range 1.11 years-12.2 years) and median body weight was 26.3 kg (range 5.9-49 kg). The
119 prevalence of the disease was between 0.007 and 0.04 per cent and higher incidences (0.04 per cent at the
120 Royal Veterinary College of London, 0.03 per cent at the University of Bristol and 0.02 per cent at the Animal
121 Health Trust) were found in southern England.

122 All dogs had a history of having travelled to or been imported from an endemic area for *Leishmania infantum*.
123 No clinical or clinicopathological differences were noted between dogs imported from and dogs that have
124 travelled to an endemic area. No autochthonous cases were found in this population. Details of patient and
125 travel history are presented in Table 1.

126

127 **Detection of *Leishmania infantum* and concurrent vector-borne diseases**

128 *Leishmania infantum* infection was demonstrated by serology and/or PCR and/or cytology. Details of the
129 diagnostic tests are shown in Table 2. Only three dogs were tested for other vector-borne diseases, including
130 two dogs tested by serology for *Ehrlichia canis* and one for *Babesia canis*. All three dogs were negative.

131

132 **Clinical signs**

133 All dogs had at least one clinical sign compatible with leishmaniasis. The most frequent reasons for
134 presentation were lethargy (20/38, 53%), dermatological manifestations (17/38, 45%), decreased appetite and
135 lameness (8/38, 21%). On physical examination the most common signs observed were dermatological signs
136 (24/38, 63%, including localised or multifocal alopecia [10], and crusting dermatitis [8]) and systemic
137 lymphadenopathy (22/38, 58%). Twenty-four per cent (9/38) of dogs were diagnosed with polyarthritis.

138

139 **Clinicopathological investigations**

140 Table 3 shows the main clinicopathological findings. All dogs had at least one laboratory abnormality
141 compatible with leishmaniasis. In total, 19/32 dogs (60%) were anaemic, with the anaemia being classified as
142 mild (haematocrit [HCT] 30-36%) and moderate (HCT 18-29%) in 11 (58%) and 8 dogs (42%), respectively,
143 and also classified as non-regenerative (reticulocytes $< 60 \times 10^9/L$) in 4 of the 6 cases where reticulocyte
144 count was available. Eight dogs (8/23, 35%) had thrombocytopenia (median: $94 \times 10^9/L$, range: 30-150;
145 laboratory reference interval: 155-400) and two (2/22, 9%) were pancytopenic. Renal azotaemia was detected
146 in 6 dogs (6/25, 24%) and 20 dogs (20/30, 67%) were classified as being in IRIS stage 1 CKD (creatinine $<$
147 $125 \mu\text{mol/l}$), 4 (4/30, 13%) in IRIS stage 2 (creatinine between $125\text{-}180 \mu\text{mol/l}$) and 6 (6/30, 20%) in IRIS
148 stage 3 (creatinine between $181\text{-}440 \mu\text{mol/l}$). None of the dogs were classified as being in IRIS stage 4 CKD
149 (creatinine $> 440 \mu\text{mol/l}$). Nineteen (19/28, 78%) of dogs were proteinuric based on increased UPCr (median:
150 5.6, range: 0.7-18.8; normal values < 0.5). Finally, 28 dogs (28/30, 93%) had hypoalbuminaemia (median: 16
151 g/l, range: 11-20, laboratory reference interval: 23-31), hyperglobulinaemia (median: 58 g/l, range: 52.1-70;
152 laboratory reference interval: 25-45) and a low (< 0.6) albumin/globulin ratio. Serum protein electrophoresis
153 was rarely used in the diagnostic work-up and/or in the follow-up rechecks.

154

155 **Treatment**

156 Of the 38 cases, 35 (92%) were given a specific treatment for CanL. In the majority of cases (17/35, 48%)
157 allopurinol was used alone, followed by a combination of allopurinol and miltefosine (15/35, 43%) or
158 allopurinol and N-methylglucamine antimoniate (3/35, 9%). A variety of other drugs were used in addition to
159 the anti-*Leishmania* therapy, depending upon the specific case and attending clinician's judgement.
160 Treatments included ace-inhibitors (benazepril, enalapril) anti-hypertensive drugs (amlodipine), anti-
161 thrombotics (clopidogrel, aspirin) analgesics and anti-inflammatory drugs (tramadol, meloxicam), gastro-
162 protectants (sucralfate, famotidine), anti-emetics (maropitant, ondansetron, metoclopramide), immune-
163 suppressive drugs (prednisolone, azathioprine), diuretics (spironolactone), antibiotics (doxycycline,
164 amoxicillin-clavulanate, enrofloxacin and marbofloxacin).

165

166 **Staging and survival**

167 Based on CLWG clinical staging, 32 dogs (32/38, 84%) were classified as stage C (sick dogs with clinically
168 evident leishmaniasis), and 6 (6/38, 16%) as stage D (severely sick dogs often unresponsive to repeated
169 courses of anti-*Leishmania* drugs). Twenty-eight (28/38, 74%) dogs were alive at the end of the study period
170 and ten (10/38, 26%) had died or had been euthanased. Six of the ten non-surviving dogs (60%) were
171 classified in stage D and 4 (4/10, 40%) in stage C. Median survival time was 400 days (range 2-2160 days).
172 Reasons for death and/or euthanasia included worsening of kidney disease (3/10, 30%), lack of response to
173 therapy (3/10, 30%), acute thrombo-embolism (1/10, 10%), neurological signs due to myelomalacia likely
174 secondary to severe systemic vasculitis (1/10, 10%) and developing of lymphoma (1/10, 10%) and
175 osteosarcoma (1/10, 10%).

176

177 **DISCUSSION:**

178 In this study data from dogs diagnosed with leishmaniasis in seven different referral centres across the UK are
179 reported. This is the first time that clinical CanL has been described in all its aspects in a population living in
180 the UK. The prevalence of the disease in this study was low demonstrating that leishmaniasis is relatively
181 uncommon in dogs living in the UK. However, the real prevalence of the disease is likely higher than the
182 current report suggests since no cases from primary practices were included. Furthermore, only dogs with
183 clinical leishmaniasis were considered, with either exposed or infected animals (those having positive results
184 to the diagnostic tests but not showing any clinical and clinicopathological abnormalities of the disease) not
185 being considered. It is unpredictable whether those dogs will develop clinical signs in the future. Moreover,
186 due to the low familiarity of the veterinary surgeons in the UK with this disease, it is possible that some cases
187 have been missed because CanL was not considered among the possible differentials. In addition, some clients
188 could have declined serology testing.

189 Unfortunately, in many cases the time-frame between the travel from/to endemic areas and the development
190 of clinical signs was not available. Anyway, it is well known that the time between the infection and the
191 development of the clinical signs (incubation period) can be very variable and mainly dependent to the host's
192 immunologic response (Fisa *et al.* 1999, Cardoso *et al.* 2007).

193 Similar to previous reports (Shaw *et al.* 2009), most cases were found in southern England. However, caution
194 should be exercised when interpreting this because no all geographical regions across the UK were included in

195 the present study. If cases from the south are genuinely overrepresented, it might be due to easier connections
196 to Europe and warmer weather. With regard to the latter, the climate has recently changed enough to support
197 the transmission and diffusion in these areas of other vector borne diseases (Medlock *et al.* 2007; Wilson *et al.*
198 2013). However, to date a vector of *L. infantum* has not been found in the UK and sand flies that are
199 introduced in the country by car or plane likely die soon after arrival due to a marked intolerance to climate
200 changes. In fact, the sand fly's range of activity is between 15° and 28°C in association with high relative
201 humidity and absence of strong rain and winds (Bogdan *et al.* 2001, Killick-Kendrick, 1999, Maroli *et al.*
202 2013). This does not rule out possible future epidemiological changes due to the ongoing global warming. To
203 date, there is little published information regarding the distribution of the competent sand fly in Northern
204 Europe and in the UK and how or if it is changing due to the warmer climate. Furthermore, other modes of
205 transmission have been described including blood transfusion (de Freitas *et al.* 2006), vertical transmission
206 from bitches to puppies (Rosypal *et al.* 2005, Pangrazio *et al.* 2009, Boggiatto *et al.* 2011, Naucke *et al.* 2012,
207 Turchetti *et al.* 2014) and venereal transmission (Diniz *et al.* 2006). Dog-to-dog mechanisms have been also
208 hypothesised to explain leishmaniasis outbreaks among foxhounds in the United States and Canada (Duprey *et*
209 *al.* 2006).

210

211 All dogs included in the present study had a history of having travelled to or been imported from a region
212 endemic for *Leishmania infantum*. The majority of dogs were imported (32/38, 84%) versus a minority that
213 has travelled to an endemic country (6/38, 16%). This would suggest a higher risk in adopting a dog from an
214 endemic area respect travelling with the dog to those countries. Travelling dogs usually stay for only a short
215 period time and the overall risk they get infected with *L. infantum* is likely low (Hamel *et al.* 2011). However,
216 veterinarians in non-endemic regions should be aware of CanL, including its non-vectorial transmission
217 modes, and should advise dog owners on preventive measures (Shaw *et al.* 2009, Menn *et al.* 2010). The
218 majority of dogs in the present study had been in Spain, which is compatible with the high prevalence of
219 leishmaniasis in this country (Mattin *et al.* 2014) and its popularity as a destination for holidays. Imported
220 shelter and stray dogs have higher risk to be infected because of decreased preventive measures and greater
221 exposure to sand flies during the period of peak of activity (evening) (Manzillo *et al.* 2006). No
222 autochthonous cases were recognised in this study, which contrasts the findings of Shaw *et al.* (2009) who

223 identified 3 positive dogs obtained from UK re-homing centres with no history of travel abroad. It remains
224 questionable if transmission was due to vectors, transplacental or even by direct contact.

225

226 The spectrum of clinical signs and laboratory abnormalities in the study group of dogs were similar to that
227 reported in endemic areas (Ciaramella *et al.* 1997, Koutinas *et al.* 1999, Paltrinieri *et al.* 2010, Solano-Gallego
228 *et al.* 2011). Dermatological signs and lymphadenopathy were the most frequent clinical findings.
229 Polyarthrititis was present in 9 dogs (24%), similarly to previously published work from the UK (Shaw *et al.*
230 2009) (17%). Polyarthrititis should be then considered among common presenting signs of leishmaniasis in
231 dogs diagnosed in UK referral centres. The most frequent clinico-pathological abnormalities found in the
232 study group included mild-to-moderate anaemia, renal azotaemia, hyperglobulinaemia, hypoalbuminaemia,
233 decreased albumin/globulin ratio and proteinuria. These are considered hallmarks of CanL also in endemic
234 areas (Ciaramella *et al.* 1997, Koutinas *et al.* 1999, Paltrinieri *et al.* 2010, Solano-Gallego *et al.* 2011).

235 Given that non-pathognomonic clinical signs and laboratory abnormalities, as well as the low familiarity with
236 the disease of the veterinary surgeons in the UK, more than one test was used to confirm the final diagnosis in
237 the majority of cases. Serology and PCR on peripheral blood was the most common combination of diagnostic
238 tests used in this population. However, PCR on peripheral blood lacks sensitivity and different tissues such as
239 lymph nodes, spleen and/or bone marrow would harbour a higher number of *Leishmania* amastigotes (Caldin
240 *et al.* 2004). Furthermore, serum protein electrophoresis was included in the initial diagnostic investigation
241 and in follow-up rechecks only in a very low number of cases. However, this test can provide important
242 information, especially during reassessment, because improvement or normalisation of the protein
243 electrophoresis trace generally happens before a negative serology titre occurs (Torres *et al.* 2011). At time of
244 diagnosis, the authors recommend the evaluation of haematology, biochemistry profile, urinalysis including
245 UPCr, serology titre and serum protein electrophoresis. In case of peripheral lymphadenopathy and/or skin
246 lesions, fine-needle aspiration for cytology and/or PCR can be also useful. After the first month of therapy,
247 previous abnormal parameters can be re-checked together with serum protein electrophoresis: in fact, as stated
248 before, this test will be the first one to show an improvement or even a normalisation of previous
249 hypoalbuminaemia and hyperglobulinaemia (usually polyclonal gammopathy). At this stage, a significant
250 reduction of the serology titre is unlikely. The latter is generally re-evaluated at 3 and 6 months from

251 diagnosis together with quantitative PCR on lymph-nodes, spleen and/or bone marrow that can demonstrate a
252 progressive reduction of the number of amastigotes.

253

254 The majority of dogs were treated with allopurinol alone, most likely because N-methylglucamine antimoniate
255 is not available in the UK and must be imported and miltefosine requires a special treatment certificate. Where
256 additional anti-*Leishmania* drugs were used, miltefosine was more frequently used than N-methylglucamine
257 antimoniate, probably because it is an oral solution and easier to administer. In contrast, N-methylglucamine
258 antimoniate must be injected subcutaneously, and can often be associated with localised pain and
259 inflammation. Anyway, both drugs have been previously shown to be similarly effective (Miró *et al.* 2009).
260 Currently, N-methylglucamine antimoniate or miltefosine in association with allopurinol are recommended as
261 standard therapy for CanL (Oliva *et al.* 2010, Solano-Gallego *et al.* 2011, Roura *et al.* 2013, Noli *et al.* 2014).
262 Some dogs also received other drugs according to the attending clinician's decision. The influence of these
263 drugs on the anti-*Leishmania* therapy and outcome is unknown.

264

265 Considering that the majority of dogs were treated only with allopurinol, it is noteworthy that the overall
266 outcome was good with a reasonable survival time. Furthermore, it should be considered that only dogs with
267 moderate-to-severe disease (stages C and D) were included in the study and that these animals generally have
268 a guarded-to-poor prognosis in endemic areas (Solano-Gallego *et al.* 2011; Roura *et al.* 2013). This finding
269 can, perhaps, be explained by the minimal chance of re-infection given the geographical location, and low risk
270 of having a concurrent vector-borne disease (Shaw *et al.* 2009). The latter cannot be completely ruled out in
271 this study population since only three dogs were tested for other vector-borne diseases. In this respect,
272 response to CanL is known to be influenced by both concurrent disease and immunological stimulation or
273 suppression by shifting the balance from a protective Th1 response to a Th2 immune response that favours the
274 development of a non-protective and possibly detrimental humoral reaction (Koutinas *et al.* 2014).

275 Most non-surviving dogs experienced a worsening of kidney disease. It is recognised that advanced renal
276 failure is the major cause of death and/or euthanasia in CanL (Panellas *et al.* 2009). Further studies evaluating
277 IRIS staging in a bigger population and also in patients already on therapy could provide more useful
278 information regarding its possible prognostic value.

279 Finally, all dogs in clinical stage D died or were euthanased. Currently clinical staging at time of diagnosis
280 and periodic re-classification in line with disease progression and regression is considered a useful way to
281 predict outcome (Solano-Gallego *et al.* 2009, Oliva *et al.* 2010, Paltrinieri *et al.* 2010).

282

283 In conclusion, although rare, veterinary surgeons in the UK should consider CanL in patients with a history of
284 travel to or from endemic areas, where there are compatible clinical signs and clinicopathological
285 abnormalities. An early diagnosis and appropriate therapy can be associated with a relatively good control of
286 the disease (Roura *et al.* 2013; Torres *et al.* 2011). As *Leishmania* infection is known to have a long
287 incubation period, practitioners should inform the owners of imported dogs to retest them for *Leishmania* for
288 at least two years after importation or in case of a clinical suspicion (Paltrinieri *et al.* 2010). Moreover,
289 veterinarians should be aware of non-vectorial transmission ways, and should advise clients on preventive
290 measures before travelling to endemic countries.

291

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Table 1: Patient population & travel history

	Number of dogs (%)
Mixed breed	12 (31%)
Labrador retriever	4 (10%)
Lurcher	3 (8%)
Cocker spaniel	2 (5%)
Golden retriever	2 (5%)
Staff bull terrier	2 (5%)
Basset hound	1 (3%)
Border collie	1 (3%)
Boxer	1 (3%)
English pointer	1 (3%)
English setter	1 (3%)
Greek hare hound	1 (3%)
Greyhound	1 (3%)
Siberian husky	1 (3%)
Labradoodle	1 (3%)
Miniature poodle	1 (3%)
Miniature schnauzer	1 (3%)
Rottweiler	1 (3%)
Spanish galgo	1 (3%)
Neutered males	18 (47%)
Neutered females	15 (40%)
Entire males	3 (8%)
Entire female	2 (5%)
Imported from	32 (84%)
Spain	16 (42%)
Greece	7 (18%)
Cyprus	3 (8%)
Italy	2 (5%)
Portugal	2 (5%)
Hungary	1 (3%)
Brazil	1 (3%)
Traveled to	6 (16%)
Spain	3 (8%)
France	2 (5%)
Germany	1 (3%)

Table 2: Diagnostic tests used to indentify *L. infantum* infection

	Number of dogs (%)
Serology + PCR	10 (26%)
Serology + PCR + Cytology	8 (21%)
Serology	7 (18%)
PCR	7 (18%)
Serology + Cytology	3 (8%)
PCR + Cytology	2 (5%)
Cytology	1 (2%)
Serology	28 (74%)
ELISA	19 (68%)
IFAT	9 (32%)
<i>Mild</i>	7 (25%)
<i>Moderate</i>	11 (39%)
<i>High</i>	10 (36%)
PCR	27 (71%)
Blood	12 (44%)
Spleen	4 (15%)
Lymph node	3 (11%)
Bone marrow	2 (7%)
Blood + Bone marrow	2 (7%)
Blood + Spleen	1 (4%)
Spleen + Lymph node	1 (4%)
Blood + Conjunctiva + Skin	1 (4%)
Blood + Bone marrow + Joint fluid	1 (4%)
Cytology	14 (37%)
Lymph node	8 (57%)
Spleen	3 (22%)
Bone marrow	2 (14%)
Lymph node + Spleen	1 (7%)